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The BCMA gene, preferentially expressed during B lymphoid maturation, is bidirectionally transcribed

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ABSTRACT

In a previous study of a t(4;16)(q26;p13) translocation, found in a human malignant T-cell lymphoma the BCMA gene, located on chromosome band 16p13.1, has been characterized. In this study we show that the BCMA gene is organized into three exons and its major initiation transcription site is located 69 nucleotides downstream of a TATA box. RNase protection assays demonstrated that the BCMA gene is preferentially expressed in mature B cells, suggesting a role for this gene in the B-cell developmental process. A cDNA complementary to the BCMA cDNA was cloned and sequenced and its presence was assessed by RNase protection assay and anchor-PCR amplification. This antisense-BCMA RNA is transcribed from the same locus as BCMA, and exhibits mRNA characteristic features, e.g. polyadenylation and splicing. It also contains an ORF encoding a putative 115 aa polypeptide, presenting no homology with already known sequences. RNase protection assays demonstrated the simultaneous expression of natural sense and antisense-BCMA transcripts in the majority of human B-cell lines tested.

INTRODUCTION

The differentiation of hematopoietic progenitor cells along the B pathway is marked by the rearrangement of variable (V), diversity (D), and joining (J) elements of immunoglobulin (Ig) genes preceding the expression of Ig heavy chain (HC) and light chain (LC) genes (1). The progenitor B (pro-B)-cells lack both HC and LC proteins, while the precursor B (pre-B)-cells produce only cytoplasmic μHC (2). Following LC gene rearrangement and expression, the pre-B-cells are converted into B-cells, which express immunoglobulin M (IgM) at their surface. Further maturation leads to Ig secreting plasma cells, which represent the final step of B-cell differentiation. While Ig gene rearrangement and expression constitute landmarks of the B-cell differentiation, other important events are likely to be required to elucidate each step of the whole process. In this regard the discovery and characterization of other genes, specifically involved in this process would help to better understand it.

We have recently reported the molecular analysis of a t(4;16)(q26;p13) translocation, observed in a human T-cell lymphoma (3). The breakpoints of both chromosome partners involved the IL2 gene on chromosome 4, and a new gene termed BCM (referred to as BCMA in the current paper) on chromosome band 16p13.1, resulting in the transcription of an hybrid IL2–BCMA mRNA composed of the three first exons of IL2 in its 5' end fused to the coding sequences of BCMA mRNA. The predicted sequence of the normal BCMA protein showed no homology with previously characterized proteins. It contains an internal 24 amino acid hydrophobic portion characteristic of a single transmembrane protein domain. A Northern blot analysis showed that the BCMA gene is normally expressed as a 1.2 kb transcript in human cell lines exhibiting mature B lymphoid cell features as well as in normal human B-cells stimulated by the Pokeweed mitogen (3). These data supported a role for BCMA in the B-cell development process. In the present report, we have characterized the exon/intron organization of the BCMA gene and the spectrum of its expression in a large panel of cells originating from different tissues using the more sensitive method of RNase protection. Our data confirmed that BCMA mRNA is mainly found in lymphoid cells undergoing B-cell differentiation. Unexpectedly, in the course of this study, we isolated cDNA clones transcribed from the opposite strand of the BCMA gene and encoding a putative 115 aa protein. Transcription of opposite DNA strands has been already reported in prokaryotic as well as in eukaryotic cell systems. While the functional mechanism of sense/antisense interactions is well established in prokaryotes, the functional significance and the mechanism of action of antisense transcripts in eukaryotes are still poorly understood.

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MATERIALS AND METHODS

Cell lines and tissues

Human precursor B-cell lines included REH (4) and JEA (5) cell lines. Other human cell lines included the KM3 lymphoid precursor cell line (6), the BL36 Burkitt lymphoma B-cell line (G.Lenoir, IARC, Lyon, France), the 167 EBV-transformed B lymphoblastoid cell line (obtained from normal cells in the lab), the IgG$^+$ LEF1 B-cell line (7), the promyelocytic NB4 cell line (8), the SUPT1 and SUPT11 (9,10) T-cell lines, the IgM$^+$ Daudi and Raji B-cell lines, the λ$^+$ RPMI 8226, the IgEλ$^+$ U266B1 cell lines, the MOLT3, MOLT4, Jurkat, Peer, DU528, HUT78, HSB2 T-cell lines and the U937 myelomonocytic cell line (American Type Culture Collection).

Nucleic acids analysis

Cloning and sequencing procedures. Genomic DNA extraction, agarose gel electrophoresis, Southern blot transfer and hybridization were performed as previously described (11). Total RNA was extracted from human organs and tissue culture cells using the guanidinium isothiocyanate method (12). Probes were labeled, using the random prime method (13) with ^{32}P-labeled nucleotides, purchased from Dupont de Nemours (Paris, France). PolyA$^+$ mRNA was prepared using oligo-dT columns. cDNA libraries were constructed with polyA$^+$ mRNAs, using cDNA synthesis kits (Pharmacia). Resulting cDNAs were ligated to *Eco*RI digested λZAPII phage arms (14) and packaged using an *in vitro* packaging kit (Amersham). Cloning procedures of recombinant phages were as previously described (11). Subcloning of genomic fragments was performed in pUC18 (15) and pBluescript (14,16) plasmid DNA digested with appropriate restriction enzymes and subsequently used for transformation of *Escherichia coli* (17). cDNA and genomic plasmid inserts were sequenced on both strands by the dideoxy chain termination procedure (18). Sequences were compared to genetic sequence data banks, GenBank (release 79) and EMBL (release 36) using GeneWorks software (IntelliGenetics, Inc.). All oligonucleotides used in this paper were purchased from Bioprobe, France. Autoradiography was performed using Kodak X-AR5 films.

RNase protection assay. Relevant cDNA restriction fragments were subcloned into pGEM-Blue plasmid vector. α-^{32}P-UTP RNA probes were synthesized from linearized DNA templates by T7 or SP6 RNA polymerase (19) using the Riboprobe II core system kit (Promega Biotech). Ten to thirty μg of test RNAs were hybridized, at 56°C overnight, with the radiolabeled antisense RNA (3×10^5 cpm) denatured for 5 mn at 90°C (20). The samples were thawed on ice, then subjected to RNase digestion with RNase A (20 μg/ml), and RNase T1 (0.7 U/ml), at 30°C for 45 mn (20). After RNase inactivation with SDS (0.6%) and proteinase K (0.3 mg/ml), samples were extracted, ethanol precipitated and then analyzed by electrophoresis through a 5% 'Hydrolink long ranger' (AT Biochemicals) polyacrylamide−7 M urea denaturing gel and autoradiography.

Isolation of cDNA clones by anchor-PCR. A modification of the SLIC (single strand ligation to single stranded cDNA) method (21) (5'-AmpliFINDER RACE kit, Clontech) was used to amplify the 5'-end of BCMA and antisense-BCMA RNAs, using 2 μg of RPMI 8226 polyA$^+$ mRNAs. BCMA first strand cDNA synthesis was performed with the 5'-CGCTGACATGTTAGAGGAGG-3' oligonucleotide (positions 592−573), while antisense-BCMA first strand cDNA synthesis was performed using the 5'-CCTCCTCTAACATGTCAGCG-3' oligonucleotide (positions 573−592). The nucleotide positions are referred to the BCMA gene nucleotide sequence (EMBL Data Library accession number Z29574). After purification and ethanol precipitation, an ampliFinder anchor was added to the 5' end of the cDNA using T4 RNA ligase. PCR amplification was carried out, under the conditions recommended by the manufacturer, with the ampliFINDER anchor primer and one of the following primers: 5'-CTGGTTCGGCCCACGAAGTTGACAAGGTATGCA-3' for BCMA and 5'-CTGGTTCGGCCCAGAACGAATGCGATTCTCTGG-3' for antisense-BCMA. Purified PCR products were cloned into the pDIRECT (Clontech) plasmid (22,23) and the mixture was used to transform competent bacterial host cells (17). Recombinant plasmids were further characterized by nucleotide sequencing.

S_1 nuclease mapping analysis. Nuclease S_1 protection experiments were performed essentially as described (20). A 400 bp fragment derived from the 5' end of the BCMA gene by cleavage with *PstI* and *Eco*RI (positions 7−408) was subcloned into the M13mp19 vector. A synthetic oligonucleotide (positions 379−340) 5'-TAGAATGTTGAGAACAATGAACTTCGCCTGCTTCGTGGGT-3' complementary to the 5' untranslated region of BCMA cDNA was end-labeled with γ-^{32}P-ATP, annealed with the single strand template DNA and extended with Klenow fragment. After digestion with *PstI* the labeled single strand probe was isolated. For the nuclease S_1 reaction, probe (5×10^4 cpm) was hybridized overnight at 30°C to 20 μg of total RNA isolated from the U266B1 human myeloma cell line and for negative controls to 20 μg of MOLT4 human T-cell line total RNA and 20 μg of yeast tRNA. The hybridization products were digested for 90 mn at 30°C with 300 U of S_1 nuclease (Boehringer) and the reaction products were analysed by electrophoresis through a 5% 'Hydrolink long ranger' (AT Biochemicals) polyacrylamide−7 M urea denaturing gel and autoradiography.

RESULTS

Genomic organization of the BCMA gene

Southern blot analysis of human placenta DNA digested by three different restriction enzymes and hybridized with a BCMA cDNA probe is shown in Figure 1. The BCMA probe detected a unique band with the three enzymes used: a 2.9 kb *Eco*RI band, a 25 kb *Bam*HI band and a 25 kb *Hind*III band. These data indicate that the BCMA gene is present as a single copy in the human genome.

We have previously isolated the 10.1 cosmid clone (3) which contains the entire BCMA coding sequence (Fig. 2A). In order to isolate and characterize the BCMA gene, we first subcloned the 5.2 kb *XbaI*−*SmaI* restriction fragment from the cosmid 10.1 and established its restriction map (Fig. 2B). We next established its entire nucleotide sequence (3802 bp) on both strands (EMBL Data Library accession number Z29574). The BCMA gene appears to be organized into three exons. Two ATG start codons are respectively located at positions 477 and 486. A TAA termination site is located at position 2993. These three exons are separated by two introns which are flanked by GT donor and AG acceptor consensus splicing sites (24). Examination of the nucleotide sequence upstream of the 5' untranslated region reveals a consensus TATA box (TATAAAT), at position 190, which

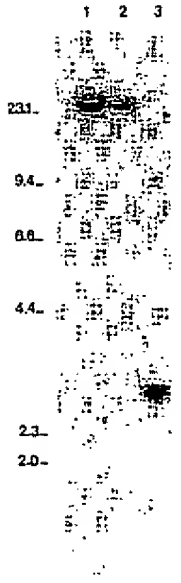

Figure 1. Southern blot analysis of human BCMA gene. Ten μg of normal human placenta DNA were digested by *BamHI* (1), *HindIII* (2) and *EcoRI* (3) restriction enzymes, electrophoresed on a 0.7 % agarose gel and transferred to nylon membrane filter. The blot was hybridized with a BCMA cDNA probe.

is a typical motif for eukaryotic polymerase II promoter genes (25–27). Examination of the nucleotide sequence immediately downstream of the 3′ untranslated region reveals a canonical AATAAA polyA signal (at position 3324) (28).

BCMA mRNA uses a non-canonical polyadenylation signal

The BCMA cDNA clones already isolated and described previously were incomplete since they lacked a polyA tail (3). In order to isolate complete BCMA cDNAs, we constructed two cDNA libraries with polyA$^+$ mRNA isolated from RPMI 8226 and U266B1 human myeloma cell lines, which have been previously reported to express BCMA mRNA (3). The screening of these two libraries with a BCMA cDNA probe resulted in the isolation of several polyadenylated cDNA clones, which were sequenced. The combination of the results obtained is depicted in Figure 3. The longest open reading frame codes for a 184 amino acid polypeptide. There are two ATG start codons in the same reading frame (positions 219 and 228) with an imperfect match to the Kozak consensus (29) and a TAG stop codon 27 nucleotides upstream of the first ATG. We found two cDNA species differing by their polyadenylation sites. One is located within an ATTAAA sequence, while the second one is located 17 nucleotides downstream of the same ATTAAA sequence. This ATTAAA polyadenylation signal is located 126 nucleotides 5′ of the canonical AATAAA signal identified in the genomic clone. However, these data do not exclude the possibility that the canonical AATAAA is functional, even if we have not cloned any BCMA cDNA using this polyadenylation signal.

BCMA transcription initiation sites

The transcription initiation sites of the BCMA gene were identified by anchor-PCR and nuclease S_1 mapping analysis. We

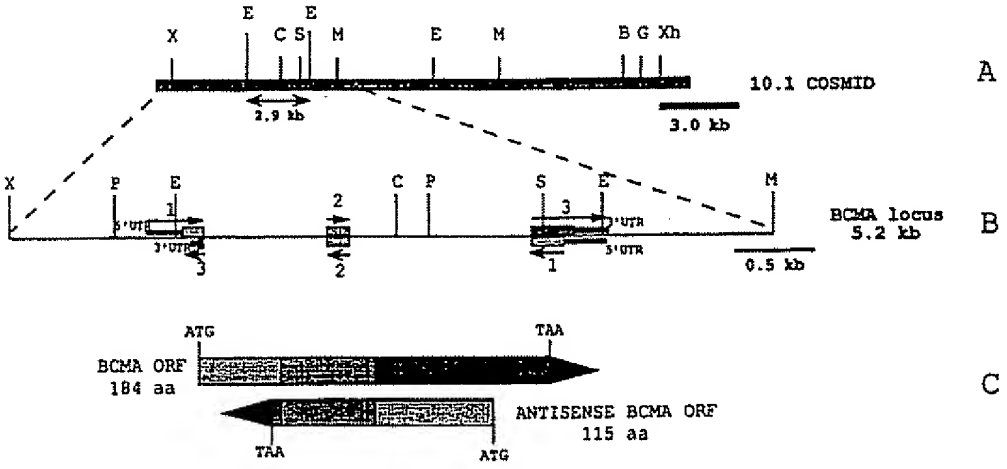

Figure 2. Organization of the human BCMA locus. A: Partial restriction map of cosmid 10.1. BCMA gene 2.9 kb *EcoRI* restriction fragment is underlined. B: Schematic representation of the BCMA locus *XbaI*–*SmaI* restriction fragment. Upper boxes correspond to BCMA gene whereas lower boxes correspond to antisense-BCMA gene. Black boxes correspond to 5′ and 3′ untranslated regions of both genes. Coding sequences are represented by shadowed boxes. Arrows show the transcription orientation of the two genes. Restriction enzymes are: X: *XbaI*, E: *EcoRI*, C: *ClaI*, S: *SalI*, M: *SmaI*, B: *BamHI*, G: *BglII*, Xh: *XhoI*, P: *PstI*. C. Schematic representation of the overlapping sense- and antisense-BCMA open reading frames. Corresponding coding exons are represented by shadowed boxes as in part B.

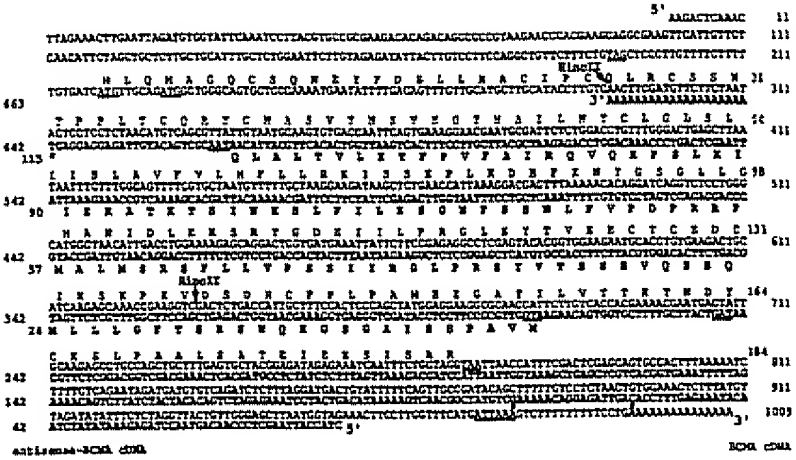

Figure 3. Nucleotide and deduced amino acid sequence of BCMA cDNA and the complementary R81 BCMA cDNA clone. On the left, the numbering of amino acids and nucleotide sequence correspond to the divergent transcript, and on the right, to BCMA cDNA. Divergent transcript and BCMA cDNA sequences are shown in bold capital and capital letters respectively. ATG start and TAA stop translation codons, upstream TAG stop codon and polyadenylation signals are underlined. PolyA sites are denoted by an asterisk. The *Hin*dII restriction fragment used in the RNase protection studies is delimited by two arrows. The nucleotide sequences have been deposited in the EMBL Data Library under the following accession numbers: Z29572 for antisense-BCMA and Z29575 for BCMA cDNAs.

first used a modified anchor-PCR method (see Materials and Methods). PCR products obtained, using polyA$^+$ mRNA from RPMI 8226 cells, were cloned into a pDirect (Clontech) cloning vector system and fourteen cDNA clones were chosen for further characterization. The longest nucleotide sequence of these clones indicated a potential transcription initiation site at position 261. Nuclease S_1 mapping detected five potential initiation sites (Fig. 4), the most upstream site is at position 259, while the other ones are located at positions 260, 261, 266 and 267. Note that the most 5' located transcription initiation site is in agreement with a consensus cap site sequence (30) located immediately upstream of this position.

Figure 4. Determination of the transcription initiation sites for human BCMA mRNA by S_1 nuclease mapping analysis. The arrowheads point to initiation sites. The sequence ladder was obtained using the same template and primer as in the synthesis of the probe. Lane 1: MOLT4 cells; lane 2: U266B1 cells; lane 3: yeast tRNA.

BCMA expression studies by RNase protection assay

We had previously shown by Northern blot analysis that the BCMA gene is transcribed as an 1.2 kb mRNA species only in mature B lymphocytes. A more accurate description of BCMA gene expression was obtained, performing RNase protection assays with RNAs from human cell lines and adult tissues. For this purpose, a *Hin*dII BCMA cDNA restriction fragment (Fig. 3) containing the major part of the BCMA coding region was subcloned, in *Sma*I digested pGEM-Blue plasmid vector. This construction was then linearized by *Eco*RI and *in vitro* transcribed by bacteriophage SP6 polymerase, resulting in a 401 nucleotide long transcript. Protection of this transcript by BCMA mRNA followed by RNase treatment gives rise to a 341 nucleotide long RNA fragment.

RNase protection assays (Table I) using 10 μg of total mRNA gave no signal, even after one month exposure, with RNAs from T-cell lines (MOLT4, Jurkat, Peer, MOLT3, DU528, HSB2, SUPT1 and HUT78), myeloid cell lines (U937, PMA-stimulated U937, NB4) and KM3 lymphoid precursor cell line. A positive signal was obtained only for the SUPT11 T-cell line. A negative result was also obtained with RNAs from several human adult tissues (brain, muscle, heart, lung, thyroid, kidney, uterus, pancreas, placenta, testis and bladder). On the contrary, BCMA transcripts were detected in lymphoid organs (spleen, lymph nodes and thymus) and also in liver and adrenals.

The results obtained using RNAs from B-cell lines representative of different stages of B-cell maturation are shown in Figure 5. BCMA mRNA was present in all B-cell lines tested (REH, JEA, Raji, BL36, LEF1, Daudi, 167, RPMI 8226 and U266B1).

Table 1. Presence of sense- and antisense-BCMA RNA transcripts in different human cell lines and human adult tissues

Cell type	Cell line	sense-RNA	antisense-RNA
Precursor lymphoid	KM3	-	+
Pre-B	REH	+	+
	JEA	-	-
B	Daudi	+	+
	Raji	+	+
	BL36	+	+
	LEF1	+	-
	167	+	+
	RPMI 8226	+	+
	U266B1	+	+
T	MOLT3	-	+
	MOLT4	-	+
	Jurkat	-	-
	Peer	-	-
	DU528	-	-
	HSB2	-	+
	HUT78	-	-
	SUPT1	-	-
	SUPT11	+	-
Myeloid	U937	-	-
	PMA-stim. U937	-	-
	HB4	-	-
Adult tissues			
Brain		-	-
Muscle		-	-
Heart		-	-
Adrenals		+	-
Lung		-	-
Liver		+	-
Thyroid		-	ND
Kidney		-	-
Uterus		-	-
Bladder		-	-
Spleen		+	-
Lymph nodes		+	-
Thymus		+	+
Pancreas		-	+
Testis		-	+
Placenta		-	ND

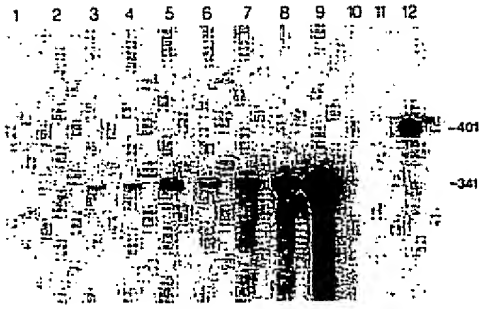

Figure 5. RNase protection analysis of the BCMA gene transcription. Ten μg of total RNA were used from MOLT4 (lane 1), REH (lane 2), JEA (lane 3), Raji (lane 4), Daudi (lane 5), BL36 (lane 6), 167 (lane 7), RPMI 8226 (lane 8) and U266B1 (lane 9) cell lines. Controls are: 20 μg of tRNA (lane 10), the RNase-digested probe (lane 11) and 8000 cpm of untreated probe (lane 12) which is 401 nucleotides long.

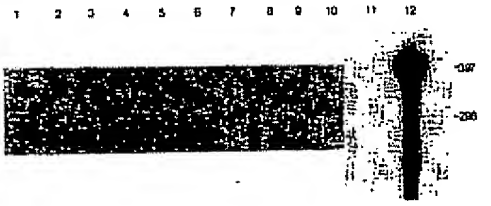

Figure 6. RNase protection analysis of the antisense-BCMA gene transcription. Thirty μg of total RNA were used from REH (lane 1), JEA (lane 2), LEF-1 (lane 3), Raji (lane 4), BL36 (lane 5), Daudi (lane 6), 167 (lane 7), RPMI 8226 (lane 8) and U266B1 (lane 9) cell lines. Controls are: 20 μg of tRNA (lane 10), the RNase treated probe (lane 11) and 8000 cpm of untreated probe (lane 12) which is 397 nucleotides long.

A natural antisense-BCMA polyadenylated RNA is expressed in B-cell lines

Screening of a RPMI 8226 cDNA library with a BCMA cDNA probe resulted in the isolation of the polyadenylated R81 clone (Fig. 3). This clone was 663 nt long and was homologous to the complementary sequence of BCMA cDNA, except for a point mutation in the 5′ UTR. To rule out the possibility that the presence of this antisense-BCMA cDNA was a cloning artifact, we performed an RNase protection study using RNAs from a panel of human B-cell lines. For this purpose, the pGEM-Blue construct previously used for the study of BCMA gene expression was linearized by *Hin*dIII and *in vitro* transcribed by bacteriophage T7 polymerase producing a 397 nucleotide long transcript. Antisense-BCMA RNA protection of this 397 nucleotide transcript results, after RNase treatment, in a 298 nucleotide long RNA fragment. The data obtained (shown in Fig. 6) clearly demonstrated the presence of an antisense-BCMA transcript in RPMI 8226, U266B1, 167, Daudi, BL36, Raji and REH B-cell lines. The fact that the positive signal obtained was very faint, even using 30 μg of total RNA and after one month exposure, indicates that the antisense-BCMA RNA is present at very low levels and is likely to explain the rarity of antisense-BCMA cDNA clones. This point should be considered when looking for a possible biological function of BCMA antisense transcripts. No signal was obtained with RNAs from JEA and LEF1 B-cell lines.

We further performed anchor-PCR amplification of antisense-BCMA RNA using an antisense-BCMA specific primer on polyA$^+$ mRNAs from RPMI 8226 cell line (see Materials and Methods). Eleven cDNA clones were obtained; the establishment of their nucleotide sequence showed their identity with the initially isolated antisense cDNA R81 clone, except for the point mutation already cited. As all these anchor PCR generated cDNA clones' sequences are identical to the genomic sequence (placenta) and originated from the same cell line (RPMI 8226) as R81 we have considered the point mutation of R81 as a sequencing artifact. Taken together, all these results clearly demonstrate the existence of a natural antisense-BCMA RNA in B-cell lines.

Expression of antisense-BCMA transcripts in other tissues

RNase protection assays using RNAs from a panel of human cell lines and human adult tissues were performed to investigate the presence of antisense-BCMA transcripts in cell lines and tissues other than those of the B lymphoid lineage. The results obtained are presented in Table 1 in comparison with those obtained for

BCMA mRNA. Antisense-BCMA RNA was detected in MOLT3 and HSB2 T-cell lines and in thymus, pancreas and testis adult tissues. On the contrary, no signal was obtained with MOLT4, Jurkat, Peer, DU528, SUPT1, SUPT11 and HUT78 mature T-cell lines and with myeloid U937, PMA-stimulated U937, NB4 cell lines and lymphoid precursor KM3 cell line. For human adult tissues no antisense-BCMA RNA was found in brain, muscle, heart, adrenals, lung, liver, kidney, uterus, bladder, spleen and lymph nodes. Taken as a whole, RNase protection studies demonstrate that a natural antisense-BCMA RNA is transcribed at low levels mostly in B-cell lines, in some T-cell lines and in some adult tissues.

Genomic organization of the antisense-BCMA gene

Since the BCMA gene is present as a unique copy in the human genome, and in view of the entire nucleotide sequence homology between R81 cDNA and BCMA cDNA, we can predict that the BCMA gene complementary strand is organized as a gene into three exons and two introns which are strictly complementary to BCMA gene exons and introns (Fig. 2). No polyA signal is found upstream or downstream the polyA addition site identified in the R81 cDNA clone. Introns 1 and 2 are strictly complementary to BCMA introns 2 and 1 respectively. Since the splicing sites of the two putative BCMA antisense gene introns are complementary to the splicing sites of the two BCMA introns, it follows that the 5' donor and the 3' acceptor sites are CT and AC respectively. These donor and acceptor sites are unusual. However, non-conforming splicing sites have already been described (24,31). It is of note that there are consensus branch point sequences for the second and the third exon located 116 nt and 72 nt upstream of the respective 3' splice site.

DISCUSSION

The BCMA gene is contained within a 3 kb segment and organized into three exons. The sequence of the exon/intron boundaries conforms to the GT/AG rule (24). Nuclease S_1 mapping analysis allowed us to detect a major BCMA transcription initiation site located immediately downstream of a consensus cap site (30). Nucleotide sequence examination of the region immediatly 5' to exon 1 reveals the existence of a consensus TATA box located 69 nucleotides upstream of the transcription initiation site.

Screening of two cDNA libraries constructed from polyA$^+$ mRNA of RPMI 8226 and U266B1 B-cell lines resulted in the isolation of two species of polyadenylated cDNAs. Both of them use an ATTAAA polyadenylation signal located 126 nt upstream the canonical AATAAA polyadenylation signal but differ in their 3' untranslated region, since two polyadenylation sites can be distinguished; the first is located within the ATTAAA sequence and the other is located 17 nt downstream of the same sequence. The ATTAAA sequence has been found to be used as a polyadenylation signal (32) by 12% of eukaryote mRNAs described up to now (33).

RNase protection assays clearly confirmed that the BCMA gene is preferentially expressed in the B-cell lineage. The BCMA gene is not transcribed in the T-cell lines tested (except in the SUPT11 post thymic T-cell line) and not at all in the myeloid cell lines used in this study. BCMA is transcribed in adult lymphoid tissues (spleen and lymph nodes) which harbor a B-cell compartment, and also in the thymus. Several studies have demonstrated that the medulla of thymus does contain a relatively mature B-cell subpopulation (34–36), which could explain our results. Alternatively this BCMA gene transcriptional activity is carried out by a non-B thymic subpopulation which remains to be identified. BCMA expression in liver and adrenals also remains to be investigated.

In the course of screening a RPMI 8226 cDNA library with the BCMA cDNA probe, we unexpectedly isolated a polyadenylated clone whose nucleotide sequence was complementary to that of BCMA cDNA. The presence of this antisense-BCMA RNA was assessed by three independent techniques: cDNA cloning, RNase protection assay and anchor-PCR. Our results showed that the antisense-BCMA is not only expressed in the RPMI 8226 cell line but also in a variety of human cell lines and tissues. Expression of antisense-BCMA appears to follow in part that of the BCMA gene, as it is expressed in almost all the B-cell lines previously shown to express BCMA (except JEA and LEF1 cell lines). The antisense-BCMA transcript was detected in two T-cell lines MOLT3 (an immature thymic cell line CD1$^+$) and HSB2 (a post thymic cell line) but not in the myeloid cell lines investigated so far. Antisense BCMA transcripts were also detected in the thymus, the pancreas and in the testis. It has to be pointed out that antisense-BCMA expression was very low compared to that of BCMA, because even by using 30 μg of RNA (three times more RNA than for BCMA detection) and five times longer film exposure the intensity of the signal obtained was faint. Estimation of the BCMA to antisense-BCMA RNA ratio was approximately 20/1 in REH cells . We concluded that the antisense-BCMA gene is expressed at low levels in the B-cell lineage in two T-cell lines and in tissues which do not express BCMA.

Because Southern blot analysis indicates that the BCMA gene exists as a single copy in the human genome, the antisense-BCMA transcripts are most likely generated from the BCMA gene opposite strand. This strand is also organized in three exons and two introns (Fig. 2) that perfectly overlap BCMA introns and exons. This organization is unusual compared to all the bidirectionally transcribed genes so far reported in which the overlap is partial and does not encompass the whole coding region. Because of this particular organization, simultaneous expression of sense and antisense transcripts could generate a situation of steric hindrance for the transcription machinery if transcripts originate from the same allele. It would be interesting to know whether the two divergent transcripts of the BCMA gene, when they are simultaneously expressed, originate or not from the same allele.

Particularly intriguing is the fact that exon/intron boundaries of the antisense-BCMA gene do not conform to the usual consensus splicing sites, as CT and AC sequences appear to represent the donor and acceptor sites respectively. Interestingly such non-conforming splicing sites have been previously described in human and murine immunoglobulin genes (31). The authors suggested that the genes containing non-conforming splicing sites might use specific components of the splicing machinery different from the usual ones.

Naturally occuring antisense RNAs have been described in prokaryotes where they have been shown to participate in the regulation of translation, transcription and/or replication of DNA (37). Bidirectional transcription with partial overlapping of the transcripts has also been reported in eukaryotes. Few of these antisense transcripts fulfill conventional criteria defining mRNAs,

e.g. polyadenylation, splicing and presence of ORFs (38–49). In spite of this large body of data there is no evidence for an effective translation of these transcripts, except for c-*myb* antisense RNA which has been shown to encode the SC35 splicing factor (50). According to some authors (47,51) SC35 might be involved in the proto-oncogene c-*myb* *trans*-splicing. The antisense-BCMA transcript exhibits the features defining an mRNA; it is spliced, polyadenylated and contains a 345 nucleotides putative open reading frame. This ORF has a coding capacity of 115 amino acids starting with an ATG initiation codon at position 272 (Fig. 3), that perfectly matches the Kozak consensus sequence (29), and ends with a TAA stop codon at position 617. An in-frame TAG stop codon is located 27 nt (position 245) upstream of the start codon suggesting that the ATG may serve as an initiator. The putative encoded polypeptide has a calculated molecular weight of 12619 Da and is rich in serine (20 of 115) and in leucine (14 of 115) residues. Neither the nucleotide nor the deduced protein antisense-BCMA sequence show an homology to currently stored sequences in databanks. Protein algorithms (52,53) predict a globular structure protein with no signal peptide cleavage consensus sequence (54). Further research (55) for known protein patterns in the PROSITE database (56) revealed four potential protein kinase C phosphorylation sites on Ser10, Ser83, Ser94 and Thr86 (57,58) and three potential *N*-glycosylation sites (59). Work is in progress to produce specific antisera in order to test whether an antisense-BCMA protein is really expressed.

Our data are also consistent with the possibility that BCMA antisense RNA is not translated and might participate in the regulation of the BCMA gene. In man, the best characterized examples of intermolecular interactions between complementary RNA sequences occur during splicing between small nuclear RNAs base pairs and complementary sequences of pre-mRNA (60). It has also been suggested that heterogeneous nuclear ribonucleoproteins (hnRNPs) may facilitate base-pairing between pre-mRNAs and regulatory antisense RNAs (61,62). Such interactions are supposed to be responsible for the inhibition, *in vitro*, of rat c-*erbA* mRNA splicing by its naturally occurring antisense RNA (63). More recently, it has been shown that the *Caenorhabditis elegans* heterochronic gene *lin-4* encodes two small RNAs (of approximately 22 and 61 nt) with antisense complementarity to the 3' UTR part of *lin-14* mRNA, suggesting that *lin-4* regulates *lin-14* translation via an antisense RNA–RNA interaction (64). Another mechanism has been described by which antisense RNA regulates the expression of a cytoplasmic basic fibroblast growth factor in fertilized *Xenopus* oocytes (43,65,66). After formation of double stranded RNA an RNA unwindase/modificase converts adenosine residues to inosine, destroying the transcript's ability to encode a functional protein. Identical enzymatic activity has been detected in human T- and B-cell lines (67). However we have not found any evidence of alterations in all the cDNAs cloned, excluding probably a role of such an RNA unwindase on the potential BCMA/antisense-BCMA RNA duplexes.

In conclusion, we have evidenced that the BCMA locus is transcribed bidirectionally into two transcripts that are preferentially co-expressed in the B-cell lineage. We have recently obtained specific antibodies raised against the BCMA protein, and we have detected BCMA polypeptide in lymphoid B-cell lines. Work is currently in progress to further characterize the BCMA protein.

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